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CHLOROPLAST DNA EVIDENCE FOR GENOME DIFFERENTIATION IN WILD POTATOES (SOLANUM SECT. PETOTA: SOLANACEAE)[1]

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Chloroplast DNA restriction site analysis has been used to test Hawkes's phylogenetic interpretations of the genomic data in *Solanum* sect. *Petota*. Hawkes hypothesized a diploid ($2n = 24$) origin of the tuber-bearing members of this group (subsection *Potatoe*) in Mexico and Central America (as a B genome) with later migrations and evolution to an A genome in South America, later followed by a return migration of the A genome to Mexico and Central America with A × B hybridizations and polyploidizations to produce ser. *Longipedicellata* (4x) and *Demissa* (6x). Our results provide partial support for this hypothesis by demonstrating the paraphyletic and primitive nature of the B genome species group, and the monophyletic and derived nature of all A genome and A × B genome species, including *S. verrucosum*, a hypothesized A genome progenitor of ser. *Demissa*. Thus, the Mexican and Central American polyploid species must have obtained their cytoplasm from the A genome. However, our results question the Stellata/Rotata hypothesis of Hawkes and the taxonomic placement of *S. chomatophilum* in ser. *Conicibaccata*.

The genus *Solanum* is by far the largest, most widespread, and most economically important genus within the Solanaceae. It includes 1,400–2,000 species found in both the Old and New Worlds, and includes one of the world's most important food crops, the potato, *Solanum tuberosum* L. D'Arcy (1972) divides *Solanum* into seven subgenera, one of which, subg. *Potatoe* (G. Don) D'Arcy, includes the potato and its immediate and more distant relatives. One section in this subgenus, sect. *Petota* Dumortier, includes the potato and its most immediate tuber-bearing (as well as a few nontuber-bearing) wild relatives. This section is further divided into subsection *Potatoe* G. Don, containing the tuber-bearing species, and subsection *Estolonifera* Hawkes which lacks them.

There is tremendous morphological and physiological variation in *Solanum* sect. *Petota*. An earlier taxonomic treatment (Correll, 1962) recognized 157 species, while the latest taxonomic interpretation (Hawkes, 1990) recognized 232 species and 22 subspecies. Eighteen of these are designated as natural hybrids, exclusive of the Mexican polyploids and five of the seven cultivated species that Hawkes (1990) believes to have resulted from hybrid speciation. The group is restricted to the Americas, occurring from the southwestern United States to southern Chile, from sea level to over 4,500 m in the South American Andes. Some species are found in the humid subtropics and some in the dry tropics (i.e., *S. calvescens* Bitt.); others in high upland alpine grasslands (puna) in tropic latitudes where they are resistant to frost (*S. acaule* Bitt.). Some occur in dry scrub or semideserts or intermontane basins (*S. chacoense* Bitt., *S. infundibuliforme* Phil.); others in high-rainfall mountain forests (*S. oxycarpum* Schiede, *S. microdontum* Bitt.). Some even occur in the dry coastal desert lomas of Peru (*S. wittmackii* Britt., *S. chancayense* Ochoa). One species (*S. morelliforme* Bitt. et Muench of Mexico and Guatemala) usually grows as an epiphyte (Hawkes, 1972, 1990).

Hawkes (1990) partitioned *Solanum* sect. *Petota* into 21 taxonomic series. Two of these series (*Etuberosa* Juz., *Juglandifolia* [Rybd.] Hawkes) lack tubers, yet have a unique combination of vegetative and floral features that influenced Hawkes (1990) to position them in a separate subsection, *Estolonifera* Hawkes. Among the remaining 19 tuber-bearing series, all in subsection *Potatoe* G. Don, Hawkes (1990) recognized two superseries: *Stellata* Hawkes (plants with stellate corollas) and *Rotata* Hawkes (with rotate corollas). Within each of these superseries he informally designated the series as primitive to more advanced

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TABLE 1. Solanum *sect.* Petota *accessions assessed for cpDNA variation*

Series/Species	PI[a]	2*n*	EBN[b]	Superseries[c]
Outgroup species				
ser. *Etuberosa* Juz.				
1. *S. brevidens* Phil.	218228	24	1	Y
Ingroup species (Mexico, Central America)				
ser. *Bulbocastana* (Rydb.) Hawkes				
2. *S. bulbocastanum* Dun.	255516	24	1	SP
3. *S. bulbocastanum*	275200	24	1	SP
ser. *Conicibaccata* Bitt.				
4. *S. agrimonifolium* Rydb.	243351	48	2	RA
ser. *Demissa* Buk.				
5. *S. demissum* Lindl.	160221	72	4	RA
6. *S. schenckii* Bitt.	498040	72	4	RA
ser. *Longipedicellata* Buk.				
7. *S. fendleri* A. Gray	497994	48	2	RA
8. *S. papita* Rydb.	251740	48	2	RA
ser. *Morelliformia* Hawkes				
9. *S. morelliforme* Bitt. et Muench	275218	24	X	SP
10. *S. morelliforme*	275223	24	X	SP
ser. *Pinnatisecta* (Rydb.) Hawkes				
11. *S. brachistotrichum* (Bitt.) Rydb.	255527	24	1	SP
12. *S. nayaritense* (Bitt.) Rydb.	518634	24	X	SP
13. *S. pinnatisectum* Dun.	275234	24	1	SP
14. *S. tarnii* Hawkes et Hjert.	498047	24	X	SP
ser. *Polyadenia* Buk. ex Correll				
15. *S. polyadenium* Greenm.	347770	24	X	SP
ser. *Tuberosa* (Rydb.)				
16. *S. verrucosum* Schldl.	310966	24	2	RA
Ingroup species (South America)				
ser. *Circaeifolia* Hawkes				
17. *S. circaeifolium* Bitt.	473461	24	1	SP
ser. *Conicibaccata*				
18. *S. chomatophilum* Bitt.	243340	24	2	RA
19. *S. colombianum* Dun.	218217	48	2	RA
ser. *Tuberosa*				
20. *S. alandiae* Cárdenas	498086	24	X	RP
21. *S. tuberosum* ssp. *andigena* (Juz. et Buk.) Hawkes	230961	48	4	RP
22. *S. chancayense* Ochoa	442699	24	1	RA

[a] USDA plant introduction numbers (see Hanneman and Bamberg, 1986).
[b] Endosperm Balance Numbers (see text); X = unknown.
[c] From Hawkes (1990). Y = undesignated, SP = primitive stellata, SA = advanced stellata, RP = primitive rotata, RA = advanced rotata.

(Hawkes, 1990), starting with ser. *Morelliformia,* a "primitive stellata" type (PS), progressing through various series exhibiting increased degrees of rotate corollas in "advanced stellata" (AS), and "primitive rotata" (PR), to, finally, "advanced rotata" (AR), represented by three South American series and the Mexican polyploid series *Conicibaccata* Bitt., *Demissa* Buk. and *Longipedicellata* Buk. (Table 1). Although this at first glance appears to place much weight on corolla morphology as a phyletic marker, Hawkes (1990) correlated corolla morphology with cytological, serological, Endosperm Balance Number (EBN, see below), crossability, and morphological data.

It has long been known and demonstrated that these wild potatoes had evolved into genomic groups, but the nature, extent, and interrelationships of these groups have been the subject of debate. These studies all involved genome analysis of intra- and interspecific hybrids and their colchicine doubled products,

and/or concomitant measures of fertility, such as pollen stainability, and seed or fruit set (Dodds, 1950; Howard and Swaminathan 1952; Swaminathan and Howard, 1953; Swaminathan and Hougas, 1954; Marks, 1955a, b, 1958, 1965, 1968; Matsubayashi, 1955, 1981; Hawkes, 1956, 1958; Pandey, 1962; Irikura, 1976). A comparison of these cytogenetic studies is complicated by different genomic formulae presented by different workers, but the following are three general points of agreement:

1. The Mexican and Central American diploid species fall into two well-differentiated genomes. One of these, designated as the B genome group, includes all members of ser. *Bulbocastana* (Rydb.) Hawkes, *Morelliformia* Hawkes, *Pinnatisecta* (Rybd.) Hawkes, and *Polyadenia* Buk. et Correll. The genome relationships of *S. clarum* (ser. *Bulbocastana*) and *S. morelliforme* (ser. *Morelliformia*) to other B genome species are equivocal. It is difficult to cross these two species to the other Mexican and Central American diploids, and they are separated from each other by chromosomal structural differences (Graham and Dionne, 1961; Marks, 1968). The second genome (designated as A) is represented by *S. verrucosum* Schlechtd. (ser. *Tuberosa* [Rybd.] Hawkes), with clear crossability relationships to the South American species and the Mexican polyploids (see below; Matsubayashi and Misoo, 1977; Matsubayashi, 1981).

2. The South American taxa, like *S. verrucosum* from Mexico, are designated as the A genome, except for five species in ser. *Etuberosa,* which are designated as the D genome (Ramanna and Hermsen, 1981). These A genome species are all very similar to each other, except for some slight differences that have been interpreted to be caused by gene substitution or cryptic structural rearrangements (Hawkes, 1972; Ramanna and Hermsen, 1979).

3. The Mexican polyploid series *Longipedicellata* (4x) and *Demissa* (6x) are allopolyploid in origin, with at least one A genome and another well-differentiated genome, designated by various authors as B in ser. *Longipedicellata* and variously as B or C in ser. *Demissa.* Hawkes (1990) reported preliminary data of L. Lopez and J. Hawkes on interspecific genome relationships within ser. *Conicibaccata* Bitt. from Mexico, Central America, and northern South America. He interpreted some intraseries genomic differentiation within the polyploid members of this series, but no firm conclusions were presented regarding intra- or interseries genome homology or relationships to other series.

The only testable hypothesis interpreting these genomic and crossability data for all of *Solanum* subsection *Potatoe* has been presented by Hawkes (1958, 1966, 1972, 1978, 1990; see Fig. 1a,b). He hypothesized a Mexican and Central American origin of the tuberous members of *Solanum* sect. *Petota,* with *S. morelliforme* as a possible extant prototype (Hawkes, 1990). The ancestral prototype species are considered to have been B genome diploid species with stellate corollas and 1EBN (see below). Subsequent dispersal of one or more of these plants to South America gradually led to the evolution of A genomes, rotate corollas, and 2EBN (and later to AA genomes and 4EBN). The evolution of the A genome in South America was then followed by a remigration of one or more of these newly evolved plants to Mexico and Central America, with hybridization and allopolyploidization with indigenous B genome species to produce series *Longipedicellata* (4x) and *Demissa* (6x). *Solanum verrucosum* of Mexico has been suggested as the extant diploid A genome contributor to ser. *Demissa,* with various members of ser. *Conicibaccata* or ser. *Longipedicellata* as the other parents (Hawkes, 1966). Extant parents have not been hypothesized for ser. *Longipedicellata* (Fig. 1a,b). The Mexican diploid species have also been hypothesized to be phylogenetically divergent because of their distinct morphology and wide interspecific variability (Correll, 1952, 1962; Hosaka, 1986a).

The present study utilizes chloroplast DNA (cpDNA) to analyze the maternal contribution of possible genomic differentiation in *Solanum* sect. *Petota,* with particular reference to the hypotheses of Hawkes (1958, 1966, 1972, 1978, 1990) regarding the geographic origin, later migrations, and hybridization of the A and B genomes. Although various authors use different genomic formulae, the designations used here are those of Hawkes (B for the Mexican diploids, A for the South American species and the Mexican diploid, *S. verrucosum,* A × B for the species of the Mexican polyploid series *Longipedicellata* and *Demissa*). The questions posed here are the following:

1. Can the B genome be demonstrated to be the primitive condition in the tuber-bearing members of *Solanum* sect. *Petota,* with the A genome species arising from within a paraphyletic B genome group?

2. Do the South American A genome species form a monophyletic group, and what are their relationships to the Mexican diploid A genome species, *S. verrucosum*?

3. What is the maternal parentage of the A × B series *Longipedicellata* and *Demissa*?

4. What are the relationships of ser. *Coni-*

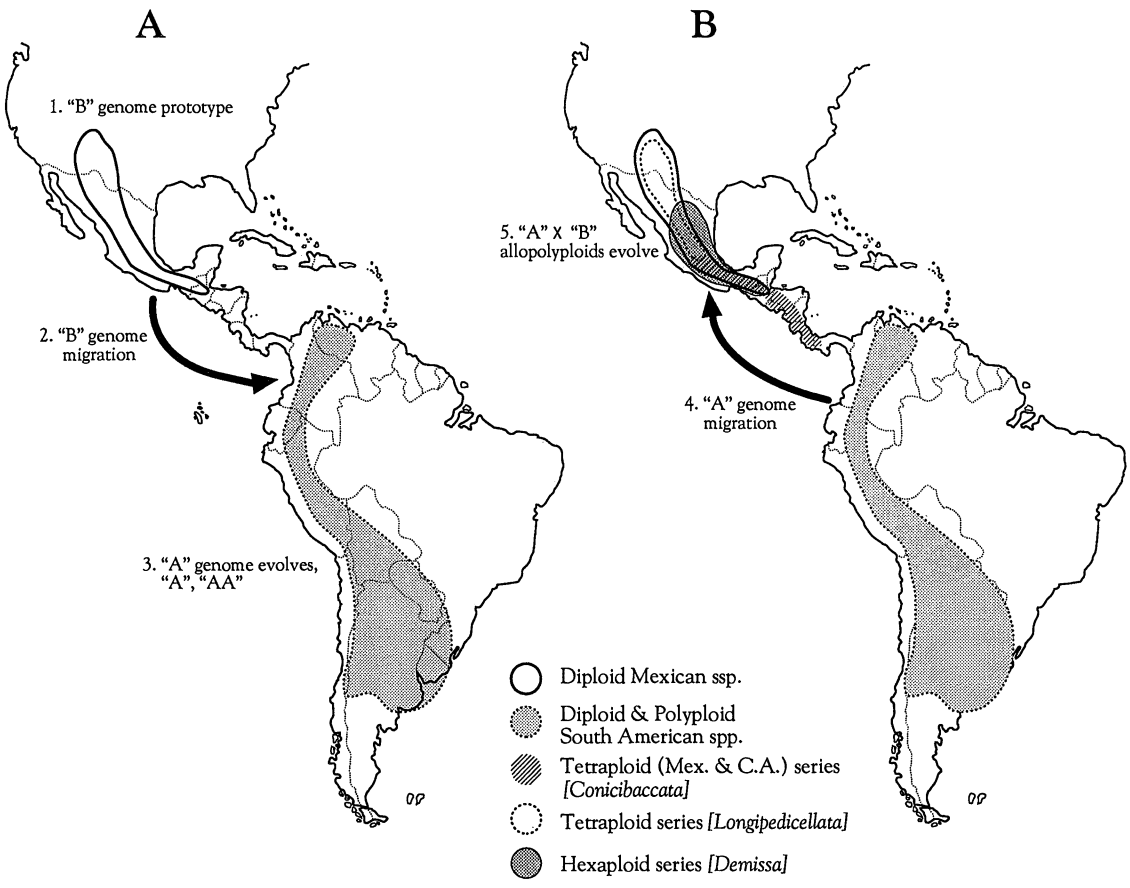

Fig. 1. Hawkes's (1990) phylogenetic/biogeographic hypothesis of genome evolution and migration in *Solanum* sect. *Petota*. A. The B genome of diploid species in Mexico and Central America represents the prototype from which a migration to South America takes place, followed by evolution to the A genome. B. A subsequent return migration to Mexico and Central America of the A genome species occurs, followed by hybridization and allopolyploidy between A and B genomes to form series *Longipedicellata* (4×) and *Demissa* (6×). The genome constitution of series *Conicibaccata* is equivocal.

cibaccata, a group of unknown genomic affinities?

5. What is the relationship of the genomically distinct *S. morelliforme* (Marks, 1968)?

6. Does cpDNA support the Stellata/Rotata evolutionary hypothesis of Hawkes (1990)?

MATERIALS AND METHODS

Plants—Seeds of 23 accessions of *Solanum* sect. *Petota*, representing all eight Mexican and Central American series, and four of the 13 South American series, were obtained from the Inter-Regional Potato Introduction Project (IR-1) in Sturgeon Bay, Wisconsin (Hanneman and Bamberg, 1986). Six of these series are confined to Mexico and Central America: *Bulbocastana, Morelliformia, Pinnatisecta, Polyadenia, Longipedicellata,* and *Demissa,* while two, *Conicibaccata* and *Tuberosa,* are represented both in this region and in South America (Table 1). The first four series are diploid ($2n = 24$), ser. *Longipedicellata* is tetraploid ($2n = 48$), ser. *Demissa* is hexaploid ($2n = 72$), ser. *Conicibaccata* is diploid, tetraploid, and hexaploid, and ser. *Tuberosa* is diploid, tetraploid, and hexaploid (including the tetraploid cultigen, *S. tuberosum*). In addition, some series contain triploid and pentaploid cytotypes (Hawkes, 1990). *Solanum brevidens,* a species of ser. *Etuberosa* native to the Andes of Argentina and Chile, was used as the outgroup. Although the outgroup relationships of *Solanum* sect. *Petota* are not fully resolved, Hawkes (1990) includes *S. brevidens* in sect. *Petota* but in a distinct subsection (subsection *Estolonifera*) separated from all of the other species here examined (subsection *Potatoe*). Additionally, Hosaka et al. (1984) provided cpDNA evidence for the outgroup status of ser. *Etuberosa.* Identifica-

TABLE 2. *Chloroplast DNA restriction site mutations within* Solanum *sect.* Petota[a]

#	Enzyme	Region	Size (kb)	Species[b]
1	*Bam* HI	P10	3.4 ↔ 2.8 + 0.6	2–22
2	*Bam* HI	P3	9.9 = 6.0 + 3.9	2, 3, 5–17, 20–22
3	*Bam* HI	P12/P14	5.9 = 3.5 + 2.4	2, 3
4	*Bam* HI	P6	7.0 = 3.5 + 3.5	19
5	*Bam* HI	T33/T34/T35	2.9 + 0.4 = 3.3	5–8, 16–22
6	*Bam* HI	P18	7.4 = 4.7 + 2.7	22
7	*Bam* HI	P16/S6	18.2 = 14.4 + 3.8	4, 19, 21
8	*Ban* I	P3	4.4 = 2.9 + 1.5	10
9	*Ban* I	S8	3.6 = 3.1 + 0.5	4 [18, 19]
10	*Ban* I	P8	1.9 + 1.4 = 3.3	9–15
11	*Ban* I	P10	2.7 + 1.3 = 4.0	2, 3
12	*Bcl* I	T33/T34/T35	4.6 ↔ 3.6 + 1.0	2–22
13	*Bcl* I	P16/S6	4.0 + 0.4 = 4.4	5–18, 20–22
14	*Bcl* I	P10	0.7 ↔ 0.5 + (0.2)	2–22
15	*Bcl* I	P6	8.1 ↔ 6.2 + 1.9	2–22
16	*Bgl* II	P10	2.8 + 1.0 = 3.8	9
17	*Bgl* II	S8	3.3 ↔ 2.3 + 1.0	2–22
18	*Bgl* II	P12/P14	0.7 = 0.4 + 0.3	9–15
19	*Bgl* II	P6	9.4 + 1.6 = 11	14
20	*Bst* NI	P8	0.9 + 0.7 = 1.6	9, 11–15
21	*Bst* NI	P3	1.1 + 0.7 = 1.8	2, 3
22	*Bst* NI	P6	1.6 ↔ 1.4 + (0.2)	2–22
23	*Bst* NI	P6	2.3 + 0.8 = 3.1	15
24	*Bst* NI	P16/S6	2.7 + 1.2 = 3.9	2, 3
25	*Bst* NI	S8	4.6 = 3.3 + 1.3	9–15
26	*Bst* NI	P16/S6	2.5 + 1.0 = 3.5	18, 22
27	*Cla* I	P3/P6	6.3 = 5.3 + 1.0	9–15
28	*Cla* I	P8	1.2 = 1.1 + (0.1)	5–8, 16, 17, 20
29	*Cla* I	P6	8.9 ↔ 5.3 + 3.6	2–22
30	*Cla* I	T36/T37/T38	6.6 + 0.8 = 7.4	15
31	*Cla* I	T36/T37/T38	7.4 = 5.3 + 2.1	2–8, 10–22
32	*Dra* I	P8/P10	0.9 + (0.1) = 1.0	9, 10
33	*Dra* I	P8	1.1 + (0.3) = 1.4	13
34	*Dra* I	P16/S6	3.8 ↔ 2.3 + 1.5	2–22
35	*Dra* I	P16/S6	1.9 = 1.7 + (0.2)	9
36	*Dra* I	P16/S6	1.7 + 1.3 = 3.0	9, 10
37	*Dra* I	P16/S6	2.7 + 0.8 = 3.5	4–8, 16–22
38	*Dra* I	P16/S6	1.6 + 1.4 = 3.0	4, 19
39	*Dra* I	P8/P10	7.9 = 6.6 + 1.3	15
40	*Dra* I	P6	12.2 = 8.7 + 3.5	2, 3, 5–8, 16, 17, 20 [9, 10, 21, 22]
41	*Dra* I	P6	6.0 + 2.7 = 8.7	18
42	*Dra* I	P3	5.0 + 0.8 = 5.8	9
43	*Dra* I	P8/P10	6.6 + 1.4 = 8.0	10
44	*Dra* I	T39/T40	1.2 + 0.9 = 2.1	4–8, 16, 17, 19, 20 [1, 21, 22]
45	*Dra* I	T39/T40	0.9 + (0.2) = 0.1	10
46	*Dra* I	T36/T37	0.5 + (0.1) = 0.6	15
47	*Dra* I	T36/T37	1.4 + (0.1) = 0.5	5–8, 16, 17, 20–22
48	*Dra* I	T38/T39/T40	3.0 + 2.0 = 5.0	4, 18, 19
49	*Dra* I	S8	4.2 + 2.5 = 6.7	9
50	*Eco* RI	P8/P10	2.1 ↔ 1.7 + 0.3	2–22
51	*Eco* RI	P6	0.7 = 0.5 + (0.2)	9–16
52	*Eco* RI	T36/T37	2.5 = 1.7 + 0.8	5–9, 17, 20
53	*Eco* RI	S8	1.6 + 0.6 = 2.2	2, 3
54	*Eco* RI	P12/P14	1.2 + 1.0 = 2.2	11
55	*Eco* RI	P3	3.6 = 3.4 + (0.2)	2, 3
56	*Eco* RV	P3	17.5 + 5.5 = 23	4–8, 14, 16–22
57	*Eco* RV	P16/S6	13.2 = 6.6 + 6.6	18
58	*Eco* RV	T39/T40	2.7 + 2.4 = 5.1	15
59	*Eco* RV	T38	11.0 = 8.0 + 3.0	18
60	*Eco* RV	S8	0.9 = 0.7 + (0.2)	2, 3, 9–15
61	*Eco* O 109	T33/T34/T35	6.4 + 0.9 = 7.3	4–8, 16–22
62	*Eco* O 109	P6	2.9 = 2.1 + 0.8	9–15
63	*Eco* O 109	P3	6.1 = 5.3 + 0.8	2, 3, 5–22
64	*Hae* II	P10/P20	17 ↔ 9.6 + 9.4	2–22

TABLE 2. *Continued*

#	Enzyme	Region	Size (kb)	Species[b]
65	*Hinc* II	P10	5.6 = 2.8 + 2.8	9
66	*Hinc* II	P6/P8	4.9 + 3.1 = 8.0	4–8, 17–22
67	*Hinc* II	P3	3.5 ↔ 2.7 + 0.8	2–22
68	*Hinc* II	P10	1.3 + 1.0 = 2.3	18
69	*Hinc* II	T38/T39/T40	0.7 + (0.5) = 1.2	2, 10
70	*Hind* III	P3	12.4 + 1.0 = 13.4	9–15
71	*Hind* III	P3	4.4 ↔ 2.9 + 1.1	2–22
72	*Hind* III	T36/T37	4.4 + 2.0 = 6.6	9–15
73	*Hpa* II	T39/T40	5.9 = 3.3 + 2.6	2, 3
74	*Hpa* II	T39/T40	1.3 + 1.3 = 2.6	9–15
75	*Hpa* II	P10	0.8 = 0.6 + (0.2)	9–15
76	*Hpa* II	P8/P10	1.6 = 1.3 + (0.3)	4–8, 16–22
77	*Hpa* II	P3	2.2 = 2.0 + (0.2)	15
78	*Hpa* II	P16/S6	2.2 ↔ 2.0 + (0.2)	2–22
79	*Hpa* II	P6	1.5 + (0.4) = 1.9	2–8, 16–22
80	*Hpa* II	S8	0.8 + (0.1) = 0.9	4–8, 16–22
81	*Hpa* II	T39/T40	0.4 + (0.3) = 0.7	15
82	*Hpa* II	T39/T40	1.7 + 1.5 = 3.2	4, 19
83	*Hph* I	P10	9.9 = 6.0 + 3.9	4–8, 16, 17, 19–21
84	*Hph* I	P10	3.1 + 0.8 = 3.9	9
85	*Hph* I	P18/P19	18 = 9 + 9	4–8, 16–21
86	*Hph* I	T39/T40	1.7 + 1.5 = 3.2	9–15
87	*Hph* I	P8	2.8 + (0.6) = 3.4	15
88	*Hph* I	P3	2.2 + 0.8 = 3.0	4, 19
89	*Hph* I	P3	3.0 = 2.5 + (0.5)	5–8, 16
90	*Hph* I	P3	2.0 = 1.2 + (0.8)	4–8, 16, 17, 19–21, [18]
91	*Nci* I	P10	5.5 = 4.9 + 0.6	9–15
92	*Nci* I	P3	3.7 + 2.3 = 6.0	15
93	*Nci* I	P6	2.7 + (0.4) = 3.1	2–8, 16–22
94	*Nci* I	S8	1.7 + (0.4) = 2.1	9–15
95	*Nci* I	T39/T40	6.9 = 4.3 + 2.6	2, 3, 10
96	*Nci* I	T39/T40	2.6 + 1.8 = 4.4	4, 19
97	*Nsi* I	P6/P8	14.7 = 10.6 + 4.1	4–8, 16–22
98	*Nsi* I	P10	8.4 = 6.6 + 1.8	12, 13
99	*Nsi* I	P10	9.4 = 8.6 + 0.8	15
100	*Rsa* I	P8	1.4 = 0.9 + 0.6	9–15
101	*Rsa* I	P3	0.6 + (0.3) = 0.9	4–9, 16–22
102	*Rsa* I	P6	5.2 = 2.7 + 2.5	4, 19
103	*Sst* I	P3	3.1 + 0.6 = 3.7	4, 19
104	*Sst* I	P10	5.1 + 0.9 = 6.0	2, 3
105	*Sst* I	P8/P10	8.9 = 6.0 + 2.9	18
106	*Sst* I	S6/S8	6.8 + 3.7 = 10.5	9–15
107	*Sst* I	P8	10.5 + 10.5 = 21	2, 3
108	*Xba* I	T38/T39/T40	1.0 + 0.5 = 1.5	4–8, 16–22
109	*Xba* I	P18/P19	2.6 = 1.4 + 1.2	4–10, 16, 17, 19–21

[a] The mutations are listed with the apomorphic state first, followed by the pleisiomorphic state (relative to *S. brevidens*). An arrow indicates that this mutation could not be polarized because *S. brevidens* differed from all other species. No site mutations were observed with *Ban* II, *Bst* XI, or *Kpn* I. Parentheses indicate where small fragments were not seen but were hypothesized to exist because length mutations were not seen with other enzymes. Brackets indicate missing data.

[b] Species numbers in Table 1.

tions used are those provided in Hanneman and Bamberg (1986); voucher specimens are deposited at the Inter-Regional Potato Introduction Project.

DNA isolation and restriction-site comparison—Pooled leaf samples of one to eight plants per accession were collected from 2-month-old plants for DNA extraction. Preparations of total DNA were made from 5 g of fresh leaf tissue by the procedure of Doyle and Doyle (1987) and purified over CsCl/ethidium bromide gradients. Restriction endonuclease digestions, agarose-gel electrophoresis, bidirectional transfer of DNA fragments from agarose gels to nylon filters, labeling of recombinant probes by nick translation, filter hybridization, and autoradiography were conducted following the methods of Palmer (1986). Twenty-two endonucleases were used to examine cpDNA

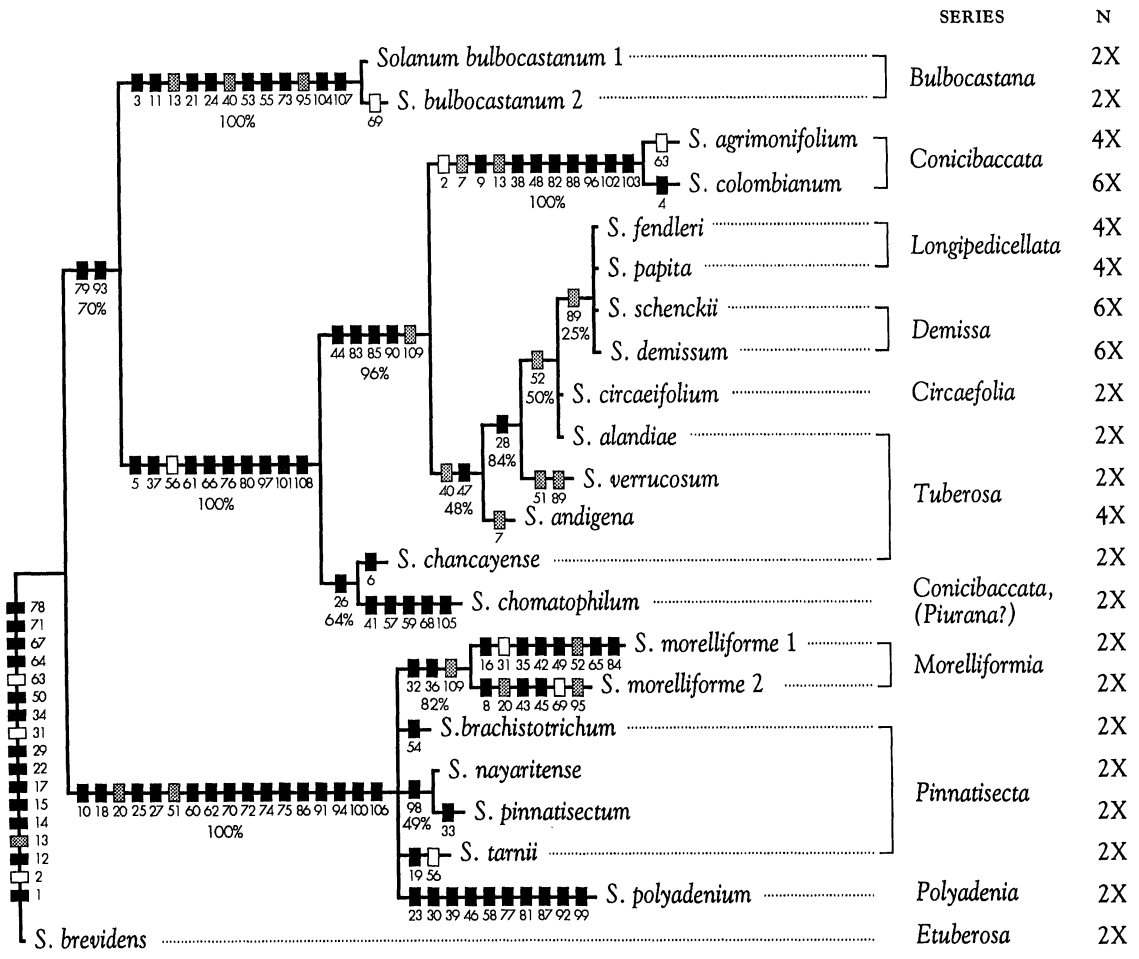

Fig. 2. One of 20 equally parsimonious 124-step Wagner trees of cpDNA restriction site mutations in *Solanum* sect. *Petota*. This tree represents the majority rule tree with 100 bootstrap replications. Bootstrap confidence values are provided for each branch. Characters are plotted onto the tree using accelerated transformation (Acctrans) option. Gray and open boxes represent homoplastic losses and gains, respectively. Species and series are abbreviated as in Table 1; numbers correspond to mutations listed in Table 2.

variation in *Solanum* (Table 2). Membranes were probed with 12 *Pst* I and two *Sal* I clones to represent nearly the entire chloroplast genome of *Petunia* (Sytsma and Gottlieb, 1986). Five clones of *Nicotiana* were used to represent the small single-copy region (courtesy of J. Palmer and R. Olmstead).

Data analysis—Restriction site data were analyzed using Wagner parsimony, which weights equally convergent site gains and convergent site losses, using PAUP (version 3.0b, D. Swofford, Illinois Natural History Survey, Urbana). The equally most parsimonious trees were sought by the branch and bound option with global branch swapping and the default accelerated transformation method (Acctrans). A consensus tree was constructed of equally parsimonious trees. The trees were rooted by outgroup comparison with *S. brevidens*. Additional trees and character distributions were examined using MacClade (version 2.97c, W. and D. Maddison). Bootstrap confidence limits (Felsenstein, 1985) on resulting branches were obtained using 100 replicates (in PAUP).

RESULTS

From a total of 109 site mutations (Table 2), 67 occurred in more than one species and thus were phylogenetically informative. Wagner parsimony produced 20 equally parsimonious 124-step trees with consistency indices of 0.879 and 0.817, with and without autapomorphies, respectively. The majority rule tree and overlayed confidence values (bootstrap) are depicted in Fig. 2, with unique mutations, homoplastic losses, and homoplastic gains in-

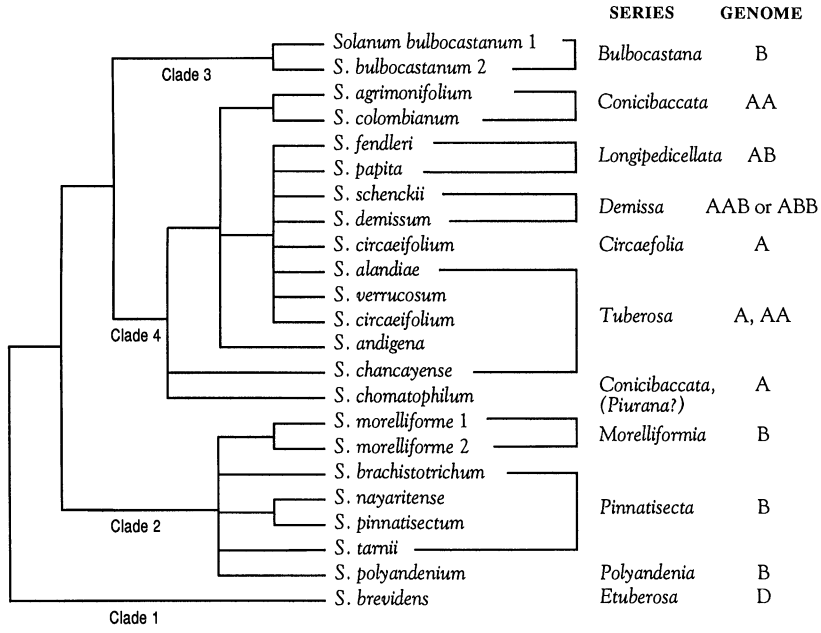

Fig. 3. The strict consensus tree of 20 equally parsimonious 124-step Wagner trees. Clades are numbered following discussion in text. Genome designations follow that of Hawkes (1990).

dicated along each branch. Of the extra 15 character changes, ten (67%) involve more likely homoplastic losses and five (33%) involve less likely homoplastic gains. Of the latter, three involve convergences with the outgroup.

A strict consensus tree of these 20 Wagner trees provides support for four main clades (Fig. 3):

1. The outgroup, *S. brevidens* (ser. *Etuberosa,* with the D genome; Ramanna and Hermsen, 1981);

2. All the Mexican diploid B genome species (Hawkes, 1978) in series *Pinnatisecta, Polyadenia,* and *Morelliformia,* exclusive of *S. bulbocastanum*;

3. *Solanum bulbocastanum* (the remaining B genome Mexican diploid, ser. *Bulbocastana*);

4. All South American species, *S. verrucosum* (the only Mexican diploid A genome species), and all Mexican polyploid species.

Resolution within some (but not all) terminal clades is poor, reflecting the low numbers of site mutations detected between closely related *Solanum* species. The amount of homoplasy for the taxa investigated is low compared to other molecular and morphological studies (Sanderson and Donoghue, 1989).

DISCUSSION

Previous cpDNA results—Hosaka et al. (1984) provided the only sectionwide cpDNA analysis of *Solanum* sect. *Petota.* Their work used 37 species within 15 taxonomic series and two additional accessions of *Lycopersicon.* They directly examined purified cpDNA, digested with eight restriction endonucleases, followed by analysis of cpDNA restriction endonuclease fragment patterns, rather than by sequential probing of smaller portions of the chloroplast genome. Four main clades were interpreted: 1. South American species, Mexican polyploids, and S. *verrucosum*; 2. Mexican diploids; 3. *S. etuberosum* (ser. *Etuberosa*); and 4. *S. lycopersicoides* (ser. *Juglandifolia*), which grouped with *Lycopersicon.*

The topology of our tree is largely in concordance with the cpDNA study of Hosaka et al. (1984), despite differences in the interpretation of mutations (Table 1, this paper; Tables 2–5, 8, Hosaka et al., 1984), and the placement of *S. bulbocastanum.* Our work extends the study of Hosaka et al. (1984), however, by the use of more B and A × B genome species, more restriction endonucleases, and by using probes rather than directly observing cpDNA banding patterns. The greater analytical power afforded by sequential probing, as done in our work, but not in previous cpDNA studies on *Solanum,* has allowed the scoring of more mutations with a greater confidence of homology. It has also allowed us to reliably distinguish insertion/deletion mutations from site mutations, and to accurately interpret densely staining bands as doublets in the single copy region from doublets in the inverted repeat region.

One significant discrepancy between our results and those of Hosaka et al. (1984) is their placement of *S. bulbocastanum* with the other Mexican B genome species. Another is their interpretation of a *Bam* HI 3.66 kb dense band in the Mexican diploids, *S. etuberosum* Lindl., *S. lycopersicoides* Dun., and *Lycopersicon* Mill. as a possible inversion relative to the remaining species. This band corresponds to our mutation 7, a site mutation found in the inverted repeat region. Similar problems in the interpretation of total cpDNA banding patterns led Hosaka (1986b) to overinterpret differences between *S. tuberosum* L. ssp. *tuberosum* and its close relatives, an error subsequently corrected by probing techniques (Hosaka, de Zoeten, and Hanneman, 1988). It is clear that accurate analysis of cpDNA in *Solanum* sect. *Petota* necessitates the use of probes, as it does in most plant groups (Palmer et al., 1988).

Cladistic relationships—Four well-defined clades are evident from this study of *Solanum* sect. *Petota* (Fig. 3):

Clade 1—*Solanum brevidens* forms a clade by default of its outgroup designation. Seventeen mutations separate this clade from all other members of sect. *Petota*. It is not possible from these data to determine those characters that are autapomorphies, but an additional analysis of *Solanum* using other outgroups indicates that over 80% of these characters represent mutations unique to ser. *Etuberosa* (D. Spooner, G. Anderson, and R. Jansen, unpublished data). The large divergence of *S. brevidens* in our study is entirely in agreement with previous genomic data (Hermsen and Taylor, 1979; Ramanna and Hermsen, 1979, 1981), morphological data (Correll, 1962; Hawkes, 1990), and chloroplast DNA results (Hosaka et al., 1984), and is associated with its inbreeding system. Most diploid species in *Solanum* sect. *Petota* are self-incompatible, the only exceptions in this analysis being all members of ser. *Etuberosa,* ser. *Morelliformia,* ser. *Polyadenia,* and *S. verrucosum* of ser. *Tuberosa* (Hawkes, 1958, 1990; Marks, 1968). Despite this wide morphological and genomic divergence, fertile interspecific hybrids have been made between members of ser. *Etuberosa* (*S. brevidens,* S. *etuberosum*) and members of both A and B genome species (*S. pinnatisectum,* B genome; *S. chacoense,* A genome [Ramanna and Hermsen, 1981; Ehlenfeldt and Hanneman, 1984; Chavez, Brown, and Iwanaga, 1988]). These results support Hawkes (1990) in including ser. *Etuberosa* in sect. *Petota,* but in a separate subsection, *Estolonifera.*

Clade 2—The Mexican B genome species, exclusive of *S. bulbocastanum,* form a well-supported clade (17 mutations, 100% bootstrap confidence). This clade is identified as the sister group of all other members of sect. *Petota* subsection *Potatoe* in all most parsimonious trees (Figs. 2, 3). The B genome group, exclusive of *S. bulbocastanum,* is shown by the cladistic analysis to be monophyletic. With *S. bulbocastanum* they are paraphyletic, and thus the A and A × B genome groups can be considered derived from a B genome group.

There are few interspecific differences within clade 2 except for two accessions of *S. morelliforme* (ser. *Morelliformia*), with six and eight autapomorphies, respectively, and one accession of *S. polyadenium* (ser. *Polyadenia*) with ten autapomorphies. There is more cpDNA differentiation within the Mexican diploids than within all other species analyzed. This cpDNA diversity correlates with greater morphological diversity of the Mexican diploid species relative to the Mexican polyploid species and the South American species (Correll, 1952, 1962; Hosaka, 1986a). *Solanum morelliforme* usually grows as an epiphyte and is morphologically distinct, although two other Mexican diploid species, *S. clarum* and *S. hintonii,* also can grow as epiphytes (Spooner et al., 1991). The series *Polyadenia* (containing *S. lesteri, S. polyadenium*) is the only Mexican series containing species with a dense indument of glandular trichomes, and like *S. brevidens* is self-compatible. The relatively large number of cpDNA autapomorphies distinguishing *S. morelliforme* and *S. polyadenium* are correlated, as in *S. brevidens,* with self-compatibility and divergent morphology (Hawkes, 1990). Our data fail to support Hawkes's (1990) hypothesis that *S. morelliforme* is "the most primitive living representative of the tuber-bearing species," indicating instead its derived status.

Clade 3—The phyletic separation of *S. bulbocastanum* from other B genome species is unexpected. *Solanum bulbocastanum* shares with members of clade 2 stellate corollas, 1EBN (Hawkes, 1990), and has genomic and crossability relationships with other Mexican and Central American B genome species (Graham and Dionne, 1961; Hawkes, 1978). The genomic data are equivocal, however. Hermsen and Ramanna (1976) show much genomic similarity, but with only small structural differences, between *S. bulbocastanum* and *S. verrucosum* (A genome), suggesting less genomic differentiation between the A and B genomes than interpreted by Graham and Dionne (1961) and Hawkes (1978). Ramanna and Hermsen

(1979) show small structural differences between *S. bulbocastanum* and *S. pinnatisectum,* another Mexican diploid, and Matsubayashi and Misoo (1977) demonstrate genomic differences between *S. bulbocastanum* and both *S. sambucinum* and *S. jamesii,* suggesting genomic differences between *S. bulbocastanum* and most other B genome diploids. Our cpDNA data conflict with that of Hosaka et al. (1984), which group all Mexican diploid B genome species together.

Clade 4—The cladistic relationship among *S. verrucosum* (2x), ser. *Longipedicellata* (4x), and ser. *Demissa* (6x), and the almost total lack of interspecific cpDNA differentiation among them suggests their common evolutionary history and recent origin, and provides partial support to the A × B genome hypothesis for the origin of ser. *Longipedicellata* and ser. *Demissa,* with *S. verrucosum* or a related species as the A genome maternal parent (see below). These results are in accordance with the genomic data (Matsubayashi and Misoo, 1979) and the cpDNA data of Hosaka et al. (1984). Some members of ser. *Conicibaccata* are supported by 11 cpDNA site mutations, and lend support to their inclusion in a separate series. Hawkes (1990, reporting unpublished data of López and Hawkes) failed to speculate on interseries genome relationships of ser. *Conicibaccata,* but indicated some genomic differences of its polyploid members. Because no diploid species of ser. *Conicibaccata* were examined in our study, we are unable to address questions of possible intraseries hybrid origins of the polyploid members.

Implications for Hawkes's genome hypothesis—This analysis provides partial support for Hawkes's hypothesis of a Mexican and Central American B genome origin of the tuber-bearing members of *Solanum* sect. *Petota,* with eventual A × B genome hybridization leading to allopolyploidy giving rise to the series *Longipedicellata* (4x) and *Demissa* (6x) (Hawkes, 1958, 1966, 1972, 1978, 1990). This hypothesis is supported by the paraphyletic nature of the B genome species and the grouping of ser. *Longipedicellata* (4x) and *Demissa* (6x) with *S. verrucosum,* a Mexican A genome diploid. The Mexican diploid B genome clade (exclusive of *S. bulbocastanum*) is shown to be the sister group of all other members of *Solanum* sect. *Petota* subsection *Potatoe* in all most parsimonious trees (Figs. 2, 3). These results indicate: 1) the paraphyly of the diploid B genome species in Mexico and Central America, 2) the derivation of the A genome species from within the B genome group, and 3) that the A × B allopolyploids of Mexico and Central America have the cytoplasm (maternal contribution) of the A genome group.

Although Fig. 3 is the most parsimonious representation of the data, the fact that only two site mutations separate clade 3 and clade 4 from clade 2 suggests an alternative cladogram, two steps longer (126 steps long). This longer cladogram unites all B genome species as a monophyletic group. A consensus tree (not shown) of the 20 most parsimonious trees and the 990 one-step longer 125-step trees exhibits an unresolved trichotomy of these three clades. However, both mutations (79, 93) supporting the separation of *S. bulbocastanum* from the other B genome diploids are site gains and therefore less likely to have been independently gained in *S. bulbocastanum,* the Mexican polyploids and the South American species (DeBry and Slade, 1985). Additionally, the 70% bootstrap confidence supporting the separation of *S. bulbocastanum* (Fig. 2) is reasonably high, considering the conservative nature of this statistic (Sanderson, 1989).

A separate cpDNA analysis (Spooner, Sytsma, and Smith, 1991) of the South American diploid species *S. canasense* Hawkes, *S. megistacrolobum* Bitt., *S. raphanifolium* Cárdenas et Hawkes, and *S. toralapanum* Cárdenas et Hawkes, used *S. bulbocastanum, S. pinnatisectum,* and *S. ochranthum* Dun. (like *S. brevidens* a member of subsection *Estolonifera*) as outgroups. The resultant cladogram places *S. pinnatisectum,* rather than *S. bulbocastanum,* as the sister group to these South American (A genome) species. Only one character, however, supports the monophyletic nature of *S. pinnatisectum* and these South American species. This discrepancy is due entirely to the selection of different outgroups and the lack of data from one enzyme (*Hpa* II) providing critical characters in the present study.

Because cpDNA is maternally inherited in *Solanum* (Hosaka et al., 1984; Corriveau and Coleman, 1988), Figs. 2 and 3 represent maternal phylogenies, and the paternal contributor to ser. *Longpedicellata* and ser. *Demissa* is thus unknown. Hawkes is the only author to have hypothesized a genome designation for the Mexican diploids, and the studies of the other authors listed above only specify genomic differences, not homology to the Mexican diploid B genome species. It is possible that the original paternal genome contributors to ser. *Longipedicellata* and ser. *Demissa* are extinct and were unrelated to the Mexican and Central American diploid B genome species. Our analysis is unable to distinguish a possible

indigenous A genome evolution in Mexico and Central America (rather than in South America), and to identify species-specific B genome contributors.

The nature of chromosome inhibition pairing (genic vs. chromosome structural) is unknown. Genome designations from meiotic analyses assume that chromosome pairing is not under genetic control, but this assumption has been questioned by Lamm (1945) and Dvořák (1983) in *Solanum* sect. *Petota*. The question of genetic control cannot be addressed by the data presented here. Because this is a cytoplasmic, and not nuclear phylogeny, it is possible that there is little correspondence between plastid and nuclear evolution, and additional molecular evidence to support Hawkes's hypothesis must await further analyses using biparentally inherited markers.

Additional phyletic insights—Hawkes (1990) designated *S. circaeifolium* Bitt. from Bolivia (ser. *Circaeifolia*) as a "primitive stellata" species and suggested that it was part of a lineage that evolved to the "advanced rotata" (including ser. *Longipedicellata* and ser. *Demissa*). We question this hypothesis on two grounds. First, the cladistic analysis places *S. circaeifolium* with advanced rotata groups in clade 4. Second, ser. *Polyadenia* species have corollas that often are more rotate than stellate (see Fig. 91, Correll, 1962; and our unpublished observations). These results, and unpublished observations of variability in the other taxa of *Solanum* sect. *Petota*, suggest that while stellate corollas may be constant in some groups, there is much intraseries variability that may limit their utility as major phylogenetic markers. Further morphological and molecular analyses of additional species, in progress, will provide a stronger test of this hypothesis.

Solanum chomatophilum Bitt. is a diploid Peruvian species of uncertain relationship. Correll (1962) and Hawkes (1963) placed it in ser. *Piurana* Bitt., but Ochoa (1962) and later Hawkes (1990) placed it in ser. *Conicibaccata*. Our results group it outside of ser. *Conicibaccata*, but only polyploid members of this series were examined here. *Solanum chomatophilum* may be truly misplaced in ser. *Conicibaccata*, or the polyploid members of this series may include a maternal parent unrelated to *S. chomatophilum*. The relationship of this species to other species within sect. *Petota* will require additional analysis including representatives from other series and analyses of the polyploid members of ser. *Conicibaccata* with biparentally inherited markers.

Some groups of species are separated by strong crossing barriers, governed by a little-understood process involving ratios of maternal/paternal genomes in the endosperm, and evidenced by endosperm breakdown (the Endosperm Balance Number or EBN hypothesis [Johnston et al., 1980; Johnston and Hanneman, 1980, 1982; Table 1]). Sexual gene transfer, however, can still be accomplished between species with different ploidy and EBN levels by means of chromosome manipulations involving haploids, $2n$ gametes, EBN numbers, bridging species, double pollinations, embryo rescue (Hermsen and Ramanna, 1973; Ehlenfeldt and Hanneman, 1984; Hanneman and Ehlenfeldt, 1984; Chavez, Brown, and Iwanaga, 1988; Peloquin, Jansky, and Yerk, 1989; Singsit and Hanneman, 1991). Clades 1, 2, and 3 all possess species with only $2n = 24$ (1EBN). Clade 4, however, is a mixture of $2n = 24$ (1EBN), $2n = 24$ (2EBN), $2n = 48$ (2EBN), $2n = 48$ (4EBN), and $2n = 72$ (4EBN), and there is no absolute relationship between chromosome number and EBN in *Solanum* sect. *Petota*.

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